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(54) Title: HUMANIZED ANTIBODIES TO CD38

(57) Abstract

The present invention relates to a monoclonal antibody, preferably with specificity for CD38, having CDRs of foreign origin and a recipient framework region having a sequence of human or primate origin, wherein the original amino acid residues in position 29 and/or 78 of the sequence of the recipient framework region of the heavy chain is replaced by a replacement amino acid residue that is the same or similar to that in the corresponding position of the sequence of the corresponding framework region of the heavy chain of the antibody from which the CDRs are derived. Method of preparation of said antibody. Pharmaceutical composition containing said antibody. Use of said antibody for the treatment of cancer and autoimmune diseases.

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Humanized antibodies to CD38

The present invention relates to antibodies and in particular to humanised antibodies and their preparation.

Antibodies typically comprise two heavy chains linked together by disulphide bonds and two light chains. Each light chain is linked to a respective heavy chain by disulphide bonds. Each heavy chain has at one end a variable domain followed by a number of constant domains. Each light chain has a variable domain at one end and a constant domain at its other end. The light chain variable domain is aligned with the variable domain of the heavy chain. The light chain constant domain is aligned with the first constant domain of the heavy chain. The constant domains in the light and heavy chains are not involved directly in binding the antibody to antigen.

The variable domains of each pair of light and heavy chains form the antigen binding site. The variable domains on the light and heavy chains have the same general structure and each domain comprises a framework of four regions, whose sequences are relatively conserved, connected by three complementarity determining regions (CDRs: CDRL1, CDRL2, CDRL3, CDRH1, CDRH2 and CDRH3). The four framework regions largely adopt a beta-sheet conformation and the CDRs form loops connecting, and in some cases forming part of, the beta-sheet structure. The CDRs are held together in close proximity by the framework regions and, with the CDRs from the other domain, contribute to the formation of the antigen binding site. The four framework regions are therefore crucial in ensuring the correct positioning of the CDRs relative to each other and hence in antibody binding.

The importance of the interaction between the CDRs and the framework regions has become increasingly evident as more and more non-human antibodies have become humanised, such humanised antibodies comprising non-human CDRs within a human framework. Humanised antibodies, in contrast to non-human antibodies, say mouse or rat antibodies, elicit a negligible immune response when administered to a human.

The prior art discloses several ways of producing such humanised antibodies. Thus EP-A-0239400 describes splicing CDRs into a human framework. Briefly, the CDRs are derived from a non-human species such as a rat or mouse whilst the framework regions of the variable domains, and the constant domains, are derived from a human antibody. Specifically, a humanised anti-CD52 antibody is disclosed in EP-A-0328404.

EP-A-054951 describes another way of humanising an antibody by re-shaping a non-human antibody to make it more like a human antibody. Basically, it comprises taking a non-human variable domain, such as mouse or rat variable domain, and changing the residues in the framework region to correspond to residues of a human framework.

In both EP-A-0239400 and EP-A-054951 an altered antibody is produced in which the CDRs of the variable domain of the antibody are derived from a first non-human species and the framework regions and, if present, the or each constant domain of the antibody are derived from human.

In such humanised antibodies a number of residues of the human framework region appear to exert a critical influence on the affinity of antigen binding (for example

Kettleborough *et al*, 1991, Prot. Eng. 4:773). Certain positions in the heavy chain framework regions, in particular, seem to be important in the retention of antigen-binding activity in a variety of altered antibodies. A number of investigators have reported the importance of residues at positions 67, 69 and 71, within the heavy chain framework region. These residues form a beta-sheet in contact with the interior aspect of the CDRH2 loop: presumably mismatches at these positions distort the CDR shape. Also, residues at positions 91 and 94 appear to be important for correct CDRH3 conformation in many heavy chains (for example Tempest *et al*, Bio/Technology 9:266). Other positions likely to affect antigen-binding are residues 27, 30 and 94 in the heavy chain, and residue 49 and 71 in the light chain (numbering according to the Kabat system). Furthermore, in the heavy chain the importance of regions 66-73 and 27-30 has been recognised in the literature, with residues 66-73 lying in close contact with CDRH2. It has now been found that the residues 29 and 78 of the framework region occupy a pocket which lies close to CDRH1 and affects antigen binding and that this undesirable effect can be obviated by using residues corresponding to those in the corresponding position of the framework region of the antibody from which the CDRs are derived.

Accordingly, the present invention is directed to a monoclonal antibody having donor CDRs of foreign origin and a recipient framework region having a sequence of human or primate origin, wherein the original amino acid residue in position 29 or 78 of the sequence of the recipient framework region of the heavy chain is replaced by a replacement amino acid that is the same or similar to that in the corresponding position of the sequence of

the corresponding framework region of the antibody from which the CDRs are derived. By "similar" is meant an amino acid of equivalent size preferably of equivalent size, hydrophobicity and charge.

Typically, the original amino acid residues in positions 29 and/or 78 of the recipient framework region are larger than their corresponding residues in the framework region of the antibody from which the CDRs are derived. Examples of these larger residues include tyrosine, histidine, tryptophan and 2-phenylalanine. Examples of the smaller corresponding residues in the framework region of the antibody donating the CDRs include glycine, alanine, valine, serine and leucine. In accordance with the invention, the larger original residue in positions 29 and/or 78 of the recipient framework is replaced with a replacement amino acid residue that is either the same or similar to the corresponding smaller residue of the antibody which is donating the CDRs.

Although it is preferable for the replacement amino acid residue to be the same as the corresponding residue of the antibody which is donating the CDRs it can also be a similar amino acid residue provided the character with respect to size and preferably also hydrophobicity and charge is essentially the same i.e. conserved. For example, if the residue of the antibody which is donating the CDRs has a valine in position 29 and/or 78, then instead of having a replacement amino acid residue in the recipient framework which is also valine, one could, for example, use alanine instead since alanine is of equivalent charge, size and hydrophobicity to valine and thus similar. The use of a similar amino acid in place of the exact same amino acid is a practice which is well established in the art and known as conservative

substitution.

By way of example, in a mouse heavy chain framework, side chains of Leu-29 and Val-78 would pack together in a small pocket close to CDRH1 whilst in the corresponding human heavy chain framework, such as for example NEW, which otherwise bears close homology to the mouse framework, the analogous positions are occupied by two Phe residues. The large aromatic side-chains appear to be too bulky to pack in the same fashion as in the mouse antibody and so alter the disposition of neighbouring surface residues resulting in a different conformation of CDRH1 in a humanised antibody. Substituting either Phe residue by the smaller murine residue partially relieves this effect allowing antigen binding. Full affinity is generally restored by replacement of both residues. It is therefore preferred that amino acids in both positions 29 and 78 are replaced.

In accordance with the invention, the replacement amino acid residues fit into the pocket without causing distortion of, for example, the CDRH1 conformation.

Preferably, the framework of the antibody heavy chain is homologous to the corresponding framework of the human antibody NEW (Saul *et al*, J. Biol. Chem. 253:585-597, 1978). The final residue of framework 1 in this case is suitably Ser or Thr, preferably Ser. This residue is at position 30 (Kabat *et al*, 1987). Preferably the framework of the antibody light chain is homologous to the variable domain framework of the protein REI (Epp *et al*, Eur. J. Biochem., 45:513-524, 1974).

Particular examples of murine heavy chains in which residues 29 and 78 pack together in a small pocket close

to CDRH1 are those in Kabat groups IB and IIC.

By contrast, other examples of human heavy chains which have bulky residues in positions 29 and 78 in the framework region are LES-C, T52, Ab44, HIgI and NEW, as listed in Kabat.

Species other than the mouse that may have residues of a small size in positions 29 and 78 are for example, the rat, rabbit and hamster.

All amino acid residue positions referred to herein employ the Kabat numbering system.

An antibody according to the invention may be produced by a method including the steps of:

(i) obtaining the sequence of a donor heavy chain;

(ii) selecting a recipient human or primate framework by best-fit homology method;

(iii) replacing the amino acid residue in position 29 or 78 of the sequence of the recipient framework region of the heavy chain by an amino acid that is the same or similar to that in the corresponding position of the sequence of the corresponding framework region of the antibody from which the CDRs are derived.

The antibody heavy chain may be co-expressed with a complementary antibody light chain. At least the framework regions of the variable domain and the or each constant domain of the complementary chain generally are derived from the primate or human recipient. Preferably

the CDRs of both chains are derived from the same selected antibody.

The antibody preferably has the structure of a natural antibody or a fragment thereof. The term antibody may therefore comprise a complete antibody, a $(Fab')_2$ fragment, a Fab fragment, Fv fragment, Fd fragment, SFv, a light chain dimer or a heavy chain and derivatives thereof. The antibody may be an IgG such as an IgG1, IgG2, IgG3 or IgG4, IgM, IgA, IgE or IgD. Furthermore, the antibody may comprise modifications of all classes e.g. IgG dimers, Fc mutants that no longer bind Fc receptors or mediate C1q binding (blocking antibodies). The antibody may also be a chimeric antibody of the type described in WO 86/01533) which comprises an antigen binding region and a non-immunoglobulin region. The antigen binding region is an antibody light chain variable domain or heavy chain variable domain. Typically, the antigen binding region comprises both light and heavy chain variable domains. The non-immunoglobulin region is fused at its C-terminus to the antigen binding region. The non-immunoglobulin region is typically a non-immunoglobulin protein and may be an enzyme, a toxin or a protein having known binding specificity. The two regions of the chimeric antibody may be connected via a cleavable linker sequence.

The invention is preferably employed to humanise an antibody, for example, an antibody of rat, rabbit, hamster or mouse origin. The framework regions and constant domains of the humanised antibody are therefore of human or primate origin whilst the CDRs of the light and/or heavy chain of the antibody are for example, rat or mouse CDRs. The antibody may be a human or primate IgG such as IgG1, IgG2, IgG3, IgG4; IgM; IgA; IgE or IgD

in which the CDRs are of rat or mouse origin.

The antibody from which the donor CDRs are derived is typically an antibody of a selected specificity. In order to ensure that this specificity is retained, either the variable domain framework regions of the antibody are re-shaped to correspond to variable domain framework regions of a human or primate antibody or the CDRs are grafted onto the closest human or primate framework regions. Either way, the resulting antibody preferably comprises non-human CDRs and human or primate framework regions that are homologous with the corresponding framework regions of the antibody from which the CDRs are derived. Preferably there is a homology of at least 50% between the two variable domains.

There are four general steps to produce a humanised antibody. These are:

(1) determining the nucleotide and predicted amino acid sequence of the light and heavy chain variable domains of the antibody from which the CDRs are derived;

(2) deciding which human or primate antibody framework region to use;

(3) the actual grafting or re-shaping methodologies/ techniques; and

(4) the transfection and expression of the grafted or re-shaped antibody.

These four steps are explained below.

Step 1: Determining the nucleotide and predicted amino acid sequence of the antibody light and heavy chain variable domains

To humanise an antibody the amino acid sequence of the non-human antibody's (donor antibody's) heavy and light chain variable domains needs to be known. The sequence of the constant domains is irrelevant. The simplest method of determining an antibody's variable domain amino acid sequence is from cloned cDNA encoding the heavy and light chain variable domain.

There are two general methods for cloning a given antibody's heavy and light chain variable domain cDNAs: (1) via a conventional cDNA library, or (2) via the polymerase chain reaction (PCR). Both of these methods are widely known. Given the nucleotide sequence of the cDNAs, it is a simple matter to translate this information into the predicted amino acid sequence of the antibody variable domains.

Step 2: Designing the humanised antibody

There are several factors to consider in deciding which human antibody (recipient antibody) sequence to use during humanisation. The humanisation of light and heavy chains are considered independently of one another, but the reasoning is basically the same.

This selection process is based on the following rationale: A given antibody's antigen specificity and affinity is primarily determined by the amino acid sequence of the variable region CDRs. Variable domain framework residues have little or no direct contribution. The primary function of the framework regions is to hold

the CDRs in their proper spacial orientation to recognise the antigen. Thus the substitution of rodent CDRs into a human variable domain framework is most likely to result in retention of the correct spacial orientation if the human variable domain is highly homologous to the rodent variable domain from which the CDRs were derived. A human variable domain should preferably be chosen therefore that is highly homologous to the rodent variable domain(s).

A suitable human antibody variable domain sequence can be selected as follows:

(i) Using a computer program, search all available protein (and DNA) databases for those human antibody variable domain sequences that are most homologous, for example, to the rodent antibody variable domains. This can be easily accomplished with a program called FASTA but other suitable programs are available. The output of the program is a list of sequences most homologous to the rodent antibody, the percent homology to each sequence, and an alignment of each sequence to the rodent sequence. This is done independently for both the heavy and light chain variable domain sequences. The above analyses are more easily accomplished if customised sub-databases are first created that only include human immunoglobulin sequences. This has two benefits. First, the actual computational time is greatly reduced because analyses are restricted to only those sequences of interest rather than all the sequences in the databases. The second benefit is that, by restricting analyses to only human immunoglobulin

sequences, the output will not be cluttered by the presence of rodent immunoglobulin sequences. There are far more rodent immunoglobulin sequences in databases than there are human.

(ii) List the human antibody variable domain sequences that have the most overall homology to the rodent antibody variable domain (from above). Do not make a distinction between homology within the framework regions and CDRs. Consider the overall homology.

(iii) Eliminate from consideration those human sequences that have CDRs that have a different length than those of the rodent CDRs. This rule does not apply to CDR 3, because the length of this CDR is normally quite variable. Also, there are sometimes no or very few human sequences that have the same CDR lengths as that of the rodent antibody. If this is the case, this rule can be loosened, and human sequences with one or more differences in CDR length can be allowed.

(iv) From the remaining human variable domains, one is selected that is most homologous to that of the rodent.

(v) The actual humanised antibody (the end result) should contain CDRs derived from the rodent antibody and a variable domain framework from the human antibody chosen above.

(vi) Instead of re-shaping or grafting to produce a humanised antibody, it would also be possible to synthesise the entire variable domain from

scratch once the amino-acids of the non-human variable domain has been determined and the most homologous human variable domain has been identified.

(vii) If donor heavy chain has two small residues at positions 29 and 78, and recipient chain has large, typically aromatic, residues at one or both of these positions, then further analysis is required.

(viii) This analysis may take the form of a sequence comparison between the CDRH1 of the donor chain and that of other antibodies. For example, a CDRH1 sequence of SYGVH has been shown to require small residues at positions 29 and 78 for complete activity, and it is to be expected that other antibodies with the same or similar CDRH1 sequence will also require residues at these positions.

Alternatively, the analysis may take the form of detailed computer aided modelling of the CDRH1 region of the proposed humanised antibody using standard techniques (for example the *AbM* package from Oxford Molecular Ltd). If this analysis, for example, reveals that CDRH1 lies in close approximation to the packed side chains of residues 29 and 78, and that altering these residues from human to smaller residues changes the orientation or position of CDRH1, then such smaller residues should replace the human ones. An example of such a perturbation of CDRH1 is shown in Figures 5 and 6.

Step 3: Grafting and re-shaping

See EP-A-0239400 and EP-A-054951 for details.

Step 4: The transfection and expression of the altered antibody

Once the antibody has been humanised and residues 29 and/or 78 replaced, the cDNAs are linked to the appropriate DNA encoding light or heavy chain constant region, cloned into an expression vector, and transfected into mammalian cells. These steps can be carried out in routine fashion. A humanised antibody may therefore be prepared by a process comprising:

(a) preparing a first replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least a variable domain of an Ig heavy or light chain, the variable domain comprising framework regions from a human or primate antibody and CDRs comprising at least parts of the CDRs from a second antibody of different origin;

(b) if necessary, preparing a second replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least the variable domain of a complementary Ig light or heavy chain respectively;

(c) transforming a cell line with the first or both vectors; and

(d) culturing said transformed cell line to produce said altered antibody.

Preferably the DNA sequence in step (a) encodes both the variable domain and the or each constant domain of the antibody chain, the or each constant domain being derived from the human or primate antibody. The antibody can be recovered and purified. The cell line which is transformed to produce the altered antibody may be a Chinese Hamster Ovary (CHO) cell line or an immortalised mammalian cell line, which is advantageously of lymphoid origin, such as a myeloma, hybridoma, trioma, or quadroma cell line. The cell line may also comprise a normal lymphoid cell, such as a B-cell, which has been immortalised by transformation with a virus, such as the Epstein-Barr virus. Most preferably, the immortalised cell line is a myeloma cell line or a derivative thereof.

Although the cell line used to produce the altered antibody is preferably a mammalian cell line, any other suitable cell line, such as a bacterial cell line or a yeast cell line, may alternatively be used. In particular it is envisaged that E. coli-derived bacterial strains could be used.

Some immortalised lymphoid cell lines, such as myeloma cell lines, in their normal state secrete isolated Ig light or heavy chains. If such a cell line is transformed with the vector prepared in step (a), it may not be necessary to carry out step (b) of the process, provided that the normally secreted chain is complementary to the variable domain of the Ig chain encoded by the vector prepared in step (a). However, where the immortalised cell line does not secrete a complementary chain, it will be necessary to carry out (b). This step may be carried out by further manipulating the vector produced in step (a) so that this vector encodes not only the variable domain of an altered

antibody light or heavy chain, but also the complementary variable domain.

Alternatively, step (b) is carried out by preparing a second vector which is used to transform the immortalised cell line. This alternative leads to easier construct preparation, but may be less preferred than the first alternative in that it may not lead to as efficient production of antibody.

Where the immortalised cell line secretes a complementary light or heavy chain, the transformed cell line may be produced for example by transforming a suitable bacterial cell with the vector and then fusing the bacterial cell with the immortalised cell line by spheroplast fusion. Alternatively, the DNA may be directly introduced into the immortalised cell line by electroporation or other suitable method.

The present process has been applied to obtain an antibody against the CD38 surface antigen.

Briefly, a humanised anti-CD38 monoclonal antibody (termed h3S) was produced in the following fashion. cDNA was obtained from hybridoma cells secreting the murine monoclonal anti-(human CD38) AT13/5. cDNA clones encoding the heavy and light chains of the mouse antibody were identified and sequenced (Sequences 1 and 2 attached in Figures 1 and 2). This information was then used to choose appropriate human frameworks to receive the CDR grafts by the best-fit homology method. This procedure identified the REI light chain and the NEW heavy chain as the optimal choices.

CDRs were grafted on to the human frameworks. In

addition, guided by published work (Riechman et al., 1988 Nature 332: 323 and Tempest et al., 1991, Bio/Technology 9:266), four framework changes were made at this stage at positions likely to affect antigen-binding: residues 27,30 and 94 in the heavy chain, and residue 49 in the light chain (numbering according to the Kabat system). The resulting humanised antibody was tested for CD38 binding, with negative results. Expression of the humanised light chain together with a chimeric heavy chain (murine VH, human CH) produced functional antibody, indicating that the humanisation of the light chain was adequate.

A further series of heavy chain framework changes were examined. In particular, the analysis identified a stretch of sequence from residue 66 to 73 which lies in close contact with CDRH2 and a pocket formed by the side chains of residues 29 and 78, lying close to CDRH1, as affecting antigen binding. As mentioned earlier on the importance of the regions 66-73 and 27-30 is recognised in the literature, though the role of residue 29 and 78 and the interaction between the side chains of residues 29 and 78 is not.

Although the invention is described with reference to an anti-CD38 antibody it is applicable to any antibody, whatever antigen it binds to. In particular any antibodies that bind the 40kD antigen (CO/17.1.A) as disclosed in J. Cell. Biol., 125 (2) 437-446, April 1994 and in Proc. Natl. Acad. Sci. 87, 3542-3546, May 1990, carcinoma antigens and antigens involved in autoimmune diseases. A specific example of an anti-40KD antibody is 323/A3.

Another example of an antibody is an anti-folate receptor

antibody as disclosed in A. Tomasetti *et al*, Federation of European Biochemical Societies Vol 317, 143-146, Feb 1993. A specific example of an anti-folate antibody is MOV18. Further examples of antibodies include anti-CEA, anti mucin, anti-20/200KD, anti-ganglioside, anti-digoxin, anti-CD4 and anti-CD23.

In particular the anti-CD38 antibody has the nucleotide sequences for the heavy chain and light chain variable region as shown in Figures 3, 3a and 4.

According to another aspect of the present invention there is provided the use of antibody according to the present invention in therapy. In particular there is provided the use of antibodies according to the invention for the treatment of cancer and their associated metastases and for treatment of autoimmune diseases, in particular for the treatment of multiple myeloma, lymphoma and rheumatoid arthritis.

The anti-CD38 antibody of the present invention can be used in the treatment of multiple myeloma.

CD38 is a transmembrane glycoprotein expressed by immature B lymphocytes, activated T and B lymphocytes, and plasma cells. Antibodies to CD38 capable of causing cell lysis may be useful in the immunotherapy of tumours bearing this antigen, principally multiple myeloma and 50% of non-Hodgin's lymphomas. Additionally, anti-CD38 antibodies may be useful in the treatment of autoimmune diseases such as rheumatoid arthritis and myaethenia gravis, as they have the potential to suppress both the humoral and cellular effector arms of the immune system.

A CD38 antibody according to the present invention has

been demonstrated to be lytic for cells expressing CD38 on their surface. The humanised antibody has been shown to bind CD38 and compete with the parental antibody in CD38 binding.

Multiple myeloma is a neoplasm characterised by an accumulation of a clone of plasma cells, frequently accompanied by the secretion of immunoglobulin chains. Bone marrow invasion by the tumour is associated with anaemia, hypogammaglobinaemia and granulocytopaenia with concomitant bacterial infections. An abnormal cytokine environment, principally raised IL6 levels, often results in increased osteoclasis leading to bone pain, fractures and hypercalcaemia. Renal failure is not uncommon in the context of high concentrations of myeloma immunoglobulin and hypercalcaemia.

A variety of therapeutic protocols have been tried over recent years with little impact on the overall prognosis for myeloma patients. Treatment with melphalan and prednisolone remains the standard therapy, as it was thirty years ago (Bergsagel, 1989). A response to chemotherapy is associated with the induction of remission with median duration of about two years, but in all cases this is followed by eventual relapse and death (Alexanian and Dimopoulos, 1994 New England J. of Medicine Vol. 330 : 484). More aggressive chemotherapy utilising multiple cytotoxic agents has yielded little additional benefit in terms of survival or duration of remission, though high-dose therapy followed by autologous bone marrow transplant remains an area of active development.

Several workers have proposed immunotherapeutic strategies to combat myeloma. Interleukin 6 has been

suggested to be a major growth factor for myeloma cells and may function in either an autocrine or paracrine fashion. Based on such results, interventions aimed at disrupting the IL6 signalling system have been designed. Two murine monoclonal that neutralise IL6 suppressed the proliferation of myeloma cells in a patient with leukaemic variant of the disease, though the tumour relapsed after 60 days.

Administration of anti-IL6 receptor monoclonal antibody to SCID mice engrafted with cells from a human myeloma cell line suppressed tumour growth, though only if the antibody was administered one day after injection of the myeloma cells. Antibody given after five days of growth had no significant effect. A CDR-grafted form of this antibody has also been prepared for possible human therapeutic use.

In a similar vein, myeloma cells bearing high levels of IL6 receptor have also been targeted by chimeric cytotoxinx consisting of IL6 variants linked to a modified form of <u>Pseudomonas</u> exotoxin. Cell killing is seen in vitro though the applicability of this technique in the clinic remains to be seen.

Our preference is for a more physiological approach, targeting myeloma cells for killing by the host immune system. The surface antigen CD38 is strongly expressed by more than 90% of multiple myeloma cells, and its suitability as a target for lytic immunotherapy has been discussed (Stevenson *et al*, 1991 Blood, Vol. 77, <u>5</u> : 1071-1079). The same report also demonstrated the competence of effector cells from myeloma patients for lysis of target cells coated with a chimeric anti-CD38.

The dosages of such antibodies will vary with the condition being treated and the recipient of the treatment, but will be in the range 1 to about 100 mg for an adult patient, preferably 1 - 10 mg, usually administered daily for a period between 1 and 30 days. A two part dosing regime may be preferable wherein 1 - 5 mg are administered for 5 - 10 days followed by 6 - 15 mg for a further 5 - 10 days.

Also included within the invention are formulation containing a purified preparation of an anti-CD38 antibody. Such formulation preferably include, in addition to antibody, a physiologically acceptable diluent or carrier possibly in admixture with other agents such as other antibodies or antibiotic. Suitable carriers include but are not limited to physiological saline, phosphate buffered saline, phosphate buffered saline glucose and buffered saline. Alternatively, the antibody may be lyophilised (freeze-dried) and reconstituted for use when needed, by the addition of an aqueous buffered solution as described above. Routes of administration are routinely parenteral including intravenous, intramuscular, subcutaneous and intraperitoneal injection or delivery.

The following Examples illustrate the invention. In the accompanying drawings:

Figure 1 shows the nucleotide and predicted amino acid sequence of mouse anti-CD38 antibody heavy chain variable region. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are underlined.

Figure 2 shows the nucleotide and predicted amino acid

sequence of mouse anti-CD38 antibody light chain variable region. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins respectively. CDRs (underlined) were identified by comparison to known immunological sequences (Kabat *et al*, "Sequences of proteins of immunologic interest", US Dept of Health and Human Services, US Government Printing Office, 1987).

Figures 3 and 3a together show the nucleotide and predicted amino acid sequence of the humanised anti-CD38 antibody light chain cDNA. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are underlined.

Figure 4 shows the nucleotide and predicted amino acid sequence of the humanised anti-CD38 antibody heavy chain cDNA. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are underlined.

Figure 5 shows the configuration of the CDRHI (dark tubes) in the murine-anti-CD38 (murine residues at positions 29 and 78).

Figure 6 shows the configuration of the CDRHI (dark tubes) in the same region as Figure 5, but in a humanised construct with human residues at positions 29 and 78.

Figure 7 shows the effect of various heavy chain framework substitutions on relative binding affinity of anti-CD38 antibodies.

Figure 8 shows the effect of various heavy chain

framework substitutions on antibody dependent cellular cytotoxicity mediated by CD38 antibodies.

Examples

Example 1

Humanisation of anti-CD38 based on a mouse antibody (AT13/5:IgGLK)

(a) General note on methodology

Unless otherwise stated, in the medodology described below, the following standard procedures and conditions were used. Manufacturers' recommended protocols were followed where applicable.

PCR experiments (Saiki *et al*, Science 239:487-491, 1988) were conducted using a programmable thermal cycler (Trio Biometra). A typical 100µl reaction mix contained 2.5 units of AmpliTaq polymerase (Perkin-Elmer Cetus, Beaconsfield, UK) in the buffer supplied by the manufacturer; 250µM of each of dATP, dCTP, DGTP and dTTP, amplification primers at 1 µM, and template DNA. Unless otherwise noted, the following cycle specifications were used:

step 0: 94°C for 90 seconds
step 1: 94°C for 60 seconds
step 2: 50°C for 60 seconds, ramping up to step 3 at a rate of 0.15°C/second
step 3: 72°C for 60 seconds, go to step 1, repeating this loop for 25 cycles
step 4: 72°C for 10 minutes.

DNA sequencing was performed by the dideoxy method using the Sequenase v2 system (USB, Cambridge, UK), according to the manufacturer's instructions. The reaction products were separated on 8% acrylamide sequencing gels (Gel-Mix 8, BRL, Paisley, Scotland, UK).

To gel-purify DNA, one of two methods was used. For fragments smaller than 175 base-pairs, the DNA was separated on a conventional high-melting point agarose gel, and the DNA recovered using the Prep-a-Gene system (Bio-Rad Laboratories, Hemel Hempstead, UK). Larger fragments were purified by separation on a low-melting point agarose gel (NuSieve GTG, FMC, Rockland, ME), and the DNA recovered using Magic PCR Preps (Promega, Southampton, UK).

Numbering of amino-acid residues in antibody chains follows the scheme of Kabat et al ("Sequences of proteins of immunological interest", US Dept of Health and Human Services, US Government Printing Office, 1991).

(b) Cloning and Sequencing of AT 13/5 antibody - Heavy Chain

Polyadenylated RNA was extracted from a culture containing 5×10^6 of the AT13/5 mouse hybridoma line using a Micro Fast Tract kit (British Biotechnology, Oxford, UK). This was converted into oligo-dT-primed single-stranded cDNA using the SuperScript Preamplification system (BRL, Paisley, Scotland, UK). Aliquots of the resulting cDNA were used in PCRs designed to separately amplify the variable region of mouse immunoglobulin heavy and light chains.

The variable region of the heavy chain was amplified

according to the method of Jones & Bendig (Bio/Technology 9:88-89), using a cocktail of primers specific to the signal peptide region (MHV1-12) and one primer specific for the mouse γ1 constant region (Mouse IgG1 heavy chain reverse primer). The resulting PCR fragment was digested with Xma I and Sal I and cloned into pUC18. Clones obtained from two independent PCR reactions were sequenced on both strands and found to be identical implying that the sequence does not contain errors introduced by the PCR process. The complete sequence of the variable region appears as Figure 1.

(c) Cloning and Sequencing of AT13/5 antibody - Light Chain

The sequence of the variable region of the light chain was also derived by a PCR-based cloning strategy using the same preparation of single-stranded cDNA as for the heavy chain. However, a more complex cloning and sequencing protocol was required, as the primers described by Jones & Bendig (*op cit*) preferentially amplify a non-productively rearranged kappa light chain from the AT13/5 cDNA. This chain arises from the fusion partner used to generate the AT13/5 hybridoma, here termed the MOPC-21 related V_K, and is of known sequence (Carroll, WL *et. al.*, Molecular Immunology 25:991-995; 1988).

To amplify the cDNA encoding the anti-CD38 light chain a PCR was performed using the mouse kappa light chain reverse primer described by Jones & Bendig (*op cit*), and a primer VK1-BACK that hybridises to the framework 1 region of most mouse kappa chains (sequences: 5' GACATTCAGCTGACCCAGTCTCCA 3'). Conditions were as described for the heavy chain amplifications above,

except that 35 cycles were used. These primers do not amplify the cDNA encoding the MOPC-21 related VK under these conditions.

An amplification fragment of the appropriate size was purified and a portion of this DNA used as the template for a second amplification (conditions as above, 30 cycles) using the light chain reverse primer and a variant of VK1-BACK containing a Hind III site (sequence: 5′ GATCAAGCTTGACATTCAGCTGACCCAGTCTCCA 3′). The resulting fragment was digested with Hind III and Xma I and cloned into a pUC18. Clones were sequenced on both strands by the conventional dideoxy method. Additionally, a portion of the PCR product was directly sequenced using a thermal cycling strategy (fmol system, Promgea, Southampton, UK) with a primer (light chain reverse primer, as above) end-labelled with ^{32}P. The sequence obtained from the cycle sequencing experiment matched exactly the sequence derived by conventional methods.

Since this sequence was obtained from the products of two rounds of amplification, further confirmation of its accuracy was sought. The existing light chain sequence was used to design a primer that hydridises to the framework 1 region (sequence: 5′ ACTAGTCGACCATCCTCCTTTTCTGTTTCTCTAGGAG 3′). This was used in conjunction with the light chain reverse primer in a PCR with the following cycle definition:

step 0: 95°C for 120 seconds
step 1: 95°C for 60 seconds
step 2: 50°C for 60 seconds
step 3: 72°C for 60 seconds, go to step 1, repeating this loop for 30 cycles
step 4: 72°C for 10 minutes

Three independent reactions were performed, and after purification, the products were digested by Xma I and Sal I, and cloned into pUC18. Several clones were sequenced by the dideoxy method. All sequences so obtained were identical to those obtained previously, confirming that the proposed light chain sequence was indeed free from PCR errors. The complete sequence of the variable region of the light chain appears as Figure 2.

(d) Design and construction of version 1 of the humanised antibody

Human variable domain frameworks were selected by the best-fit homology method (Lewis, AP & Crowe, JS in "Generation of Antibodies by Cell and Gene Immortalisation", Terhorst, C, Malavasi, F, Albertini, A (eds) Karger: Basel, 1993). The frameworks chosen for humanisation process were the light and heavy chain variable domains of Campath 1H (disclosed in EP-A-0328404). The humanised heavy and light chains were then constructed by a recombinant PCR technique (Lewis & Crowe, Gene 101:297-302, 1991).

i) Light Chain

The primers used in the humanisation process were:

A_L:5'GATCAAGCTTCTCTACAGTTACTGAGCACA3'
B_L:5'CCGATTATATATGTCCTCACTTGCCTTACAGGTGATGGTCAC3'
C_L:5'AGTGAGGACATATATAATCGGTTAACCTGGTACCAGCAGAAG3'
D_L:5'AGTTTCCAAACTGGTTGCACCAGAGATCAGCAGCTTTGG3'
E_L:5'GGTGCAACCAGTTTGGAAACTGGTGTGCCAAGCAGA3'
F_L:5'GTACGGATTACTCCAATACTGTTGGCAGTAGTAGGTGGC3'
G_L:5'CAGTATTGGAGTAATCCGTACACGTTCGGCCAAGGGACC3'
H_L:5'GATCAAGCTTCTAACACTCTCCCCTGTTGA3'

Primers A_L and H_L contain Hind III sites to allow cloning of the final amplficiation product. PCRs were performed according to the following cycle specification:

step 0: 95°C for 120 seconds
step 1: 95°C for 60 seconds
step 2: 45°C for 60 seconds
step 3: 72°C for 60 seconds, go to step 1, repeating this loop for 25 cycles
step 4: 72°C for 10 minutes

The template used in this reaction was DNA encoding the Campath 1H light chain, a construct in which the framework residues are taken from REI and the CDRs from a rat anti-human CDw52 antibody (Reichmann, L. *et. al.* Nature 332:323-337, 1988). The primers above are designed to wholly replace the Campath 1H sequence, leaving the AT13/5 CDRs grafted onto the REI frameworks.

Four initial PCRs were performed using 10ng of template with the primer pairs: A_L and B_L, C_L and D_L, E_L and F_L, and G_L and H_L. The products of these reactions, AB_L, CD_L, EF_L and GH_L respectively were gel-purified and half of the amount recovered used in the second round of PCRs. Fragments AB_L and CD_L were used as template with primers A_L and D_L in one reaction, and fragments EF_L and GH_L were used as template with primers E_L and H_L. The reacton conditions were:

step 0: 95°C for 120 seconds
step 1: 95°C for 60 seconds
step 2: 45°C for 60 seconds
step 3: 72°C for 90 seconds, go to step 1, repeating this loop for 20 cycles

The products of these reactions, AD_L and EH_L, were gel-purified and half of each DNA used as template in a final reaction with primers A_L and H_L with the reaction conditions as for the second round PCR above. The resulting product was digested with Hind III and cloned into pUC18. A clone with the predicted structure as determined by complete sequence of the insert on both strands was chosen for further manipulation. The sequence of the variable region of this construct is given as Figures 3 and 3a.

ii) Heavy Chain

The primers used in the humanisation process were:

A_H: 5'GATCAAGCTTTACAGTTACTCAGCACACAG3'
B_H: 5'GTGGACACCATAACTGGTGAAGGTGAAGCC3'
C_H: 5'AGTTATGGTGTCCACTGGGTGAGACAGCCA3'
D_H: 5'TTGTAGTCTGTGCTTCCACCTCTCCACATCACTCCAATCCACTCAAG3'
E_H: 5'GAAGCACAGACTACAATGCAGCTTTCATGTCCAGAGTGACAATGCTG3'
F_H: 5'GGAGTCCATCACGAAGCCGGTCGTTATCATGGATTTTGCACAATAATAGAC3'
G_H: 5'AAATCCATGATAACGACCGGCTTCGTGATGGACTCCTGGGGTCAAGGCTCACTAGTCACAGTCTCCTCAGCC3'
H_H: 5'TAGAGTCCTGAGGGAATTCGGACAGCCGGGAAGGTG3'

PCRs were performed according to the following cycle specification:

step 0: 95°C for 120 seconds
step 1: 95°C for 60 seconds
step 2: 45°C for 60 seconds
step 3: 72°C for 60 seconds, go to step 1, repeating this loop for 25 cycles
step 4: 72°C for 10 minutes

The template used in this reaction was DNA encoding the Campath 1H heavy chain, a construct in which the CDRs and framework residues 27 and 30 are taken from a rat anti-human CDw52 antibody (Reichmann, L *et. al.* op cit), and the remainder of the framework residues from NEW. The primers above are designed to replace the Campath 1H CDR sequences, leaving the AT13/5 CDRs grafted onto the Campath 1H framework. Also, heavy chain residue 94 is known to be important in antigen-binding (Tempest, PR *et. al.*, Bio/Technology, 9:260-271, 1991), so the AT13/5 sequence was adopted at this position. The rat sequence at residues 27 and 30 is more homologous to the AT13/5 sequence than is the unmodified NEW sequence. Primers A_H and H_H contains Hind III and EcoR I sites respectively. Additionally, primer G_H engineers a SpeI site into the framework 4 region to allow coupling to a previously prepared human C_H sequence.

Four initial PCRs were performed using 10ng of template with the primer pairs: A_H and B_H, C_H and D_H, E_H and F_H, and G_H and H_H. The products of these reactions, AB_H, CD_H were used as template with primers A_H and D_H in one reaction, and fragments EF_H and GH_H were used as template with primers E_H and H_H. The reaction conditions were:

step 0: 95°C for 120 seconds
step 1: 95°C for 60 seconds
step 2: 45°C for 60 seconds
step 3: 72°C for 90 seconds, go to step 1, repeating this loop for 20 cycles

The products of these reactions, AD_H and EH_H, were gel-purified and half of each DNA used as template in a final reaction with primers A_H and H_H with the reaction conditions as for the second round PCR above. The

resulting product was digested with Hind III and Spe I, and the fragment containing the variable region cloned into a pUC18-based vector containing the human C_H sequence. A clone with the predicted structure as determined by complete sequencing of the insert on both strands was chosen for further manipulation.

(e) Eukaryotic expression of version 1 of the humanised antibody

Humanised AT13/5 heavy and light chains were cloned into eukaryotic expression vectors under human β actin promoters. The heavy and light chain plasmids were transiently expressed in B11 CHO cells by cotransfection of the two plasmids using Transfectam (Promega, Southampton, UK). Culture supernatants were assayed for human IgG by ELISA, and tested for CD38-binding activity by FACS analysis using the CD38-positive B-cell line Wien 133.

Although the culture supernatants contained significant amounts of human IgG, no anti-CD38 activity could be detected by FACS, even when supernatants were concentrated 10-fold. This result suggests that simple grafting of the CDRs from AT13/5 onto the Campath 1H and REI human frameworks is insufficient to transfer the antibody specificity. A series of framework changes were therefore undertaken in order to restore CD38-binding activity.

(f) Framework changes

Since most of the framework residues previously shown to be important in restoring antigen binding are in the heavy chain variable region, it was decided to focus on

this part of the antibody. Additional cotransfection of the humanised light chain with a chimaeric heavy chain construct (mouse heavy variable region fused to human C_H), produced active antibody (hereafter termed hybrid antibody) that bound CD38 with an affinity comparable to that of the original mouse antibody. The region with the lowest homology between the human frameworks used and the original mouse sequence is also close to some residues of known importance. This region, just downstream of the CDR3 sequence was chosen for mutagenesis.

Heavy chain residues 67 to 71 inclusive and 73 were grafted from the mouse antibody onto the humanised heavy chain using recombinant PCR. The primers used were as follows:

A_H: sequence as above
I_H: 5'GTTGTCCTTGGTGATGTTCAGTCTGGACATGAAAGCTGC3'
J_H: 5'CTGAACATCACCAAGGACAACAGCAAGAACCAGTTCAGC3'
H_H: sequence as above.

Two initial PCRs were performed using 10ng of version 1 humanised heavy chain template with the primer pairs: A_H and I_H and J_H and H_H. The products of these reactions, AI_H and JH_H respectively, were gel-purified and half of the recovered DNA used in a second round of PCR with primers A_H and H_H to generate version 2 of the humanised heavy chain variable region. This was cloned, sequenced, transferred to the expression system, and then transiently co-expressed with the humanised light chain construct as above. Once again, culture supernatant from transfected CHO cells produced human IgG as determined by ELISA, but no CD38-binding activity could be detected by FACS analysis.

A further round of mutations based on both version 1 and version 2 of the humanised heavy chain were then produced by a method identical to that described above. A total of six version 3 heavy chains were produced in which the following heavy chain framework residues were grafted from the mouse sequence onto one or other humanised sequence:

Antibody	Template for mutagenesis	Grafted residues	Primers used
h3J	version 1	28,29	K_H, L_H
h3K	version 2	28,29	K_H, L_H
h3L	version 1	76	M_H, O_H
h3M	version 2	76	N_H, O_H
h3N	version 1	28,29,76	K_H, L_H, M_H, O_H
h3O	version 2	28,29,76	K_H, L_H, N_H, O_H

Additionally, all constructions used primers A_H and H_H. The primer sequences used were:

A_H: sequence as above
H_H: sequence as above
K_H: 5'ACTGGTTAACGAAAAGCCAGACACGGTGCAGGTCAG3'
L_H: 5'GGCITTTCGTTAACCAGTTATGGTGTCCACTGGGTG3'
M_H: 5'AAATTGCCGTTTCGAAGTGTCTACCAGCATTGTCAC3'
N_H: 5'AAATTGCCGTTTCGAATTGTCCTTGGTGATGTTCAG3'
O_H: 5'TTCGAAACGGCAATTTAGCTTGAGACTCAGCAGC3'

Heavy chain constructs containing the expected sequence were transferred into mammalian expression vectors, and cotransfected with the humanised light chain construct into CHO cells, as above. Tissue culture supernatants containing human IgG as determined by ELISA were assayed for CD38-binding activity by FACS. Constructs h3K and h3O showed antigen-binding in this assay though with less

activity than the hybrid antibody (see Fig. 7).

(g) Method for changing framework residues at positions 29 and 78

In order to establish why h30 showed less activity than the hybrid antibody further sequences analysis suggested potential problems with positions 29 and 78 in the heavy chain.

Having identified mutations to be made in the heavy chain framework regions, these can be produced by a variety of standard methods: examples being site-directed mutagenesis, recombinant PCR and gene synthesis using oligonucleotides. In the case of the anti-CD38 heavy chain VH, recombinant PCR was used to introduce murine residues at positions 28-29 and 78 sequentially.

A human anti-CD38 heavy chain VH already incorporating murine residues at positions 27, 30, 67, 68, 69, 70, 71, 73 and 94 (Version 2 as described in (f) above) was used as template for the first round of mutagenesis. This was amplified with the following PCR primers in two separate reactions:

Primer A: 5'GATCAAGCTTTACAGTTACTCAGCACAG3'
Primer B: 5'ACTGGTTAACGAAAAGCCAGACACGGTGCAGGTCAG3'
Primer C: 5'GGCTTTTCGTTAACCAGTTATGGTGTCCACTGGGTG3'
Primer D: 5'TAGAGTCCTGAGGGAATTCGGACAGCCGGGAAGGTG3'

In primers B and C, the triplets encoding the murine residues at positions 28 and 29 are underlined. In the first reaction, the template was amplified with primers A and B. In the second reaction, the template was amplfified with primers C and D. The products of the two reactions were purified, mixed, and amplified with primers A and D. The reaction product was purified,

cleaved with Hind III and SpeI, and the 450 base-pair fragment encoding the VH cloned into a variant of pUC18 containing a human γ1 cDNA cassette (Sime *et al*, 1993; J. Immunol, 151:2296). Clones were sequenced to ensure correct introduction of the murine residues at positions 28 and 29.

A clone incorporating these changes was then used as template for a second round of recombinant PCR mutagenesis to introduce the murine residue at position 78. A procedure identical to that described above was followed, except that primers B and C were replaced by primers E and F respectively, which contain a triplet (underlined) that incorporates the murine residue at position 78.

Primer E: 5'AACCAG<u>GTG</u>AGCTTAAGACTCAGCAGCGTGACA3'
Primer F: 5'TCTTAAGCT<u>CAC</u>CTGGTTCTTGCTGTTGTCCTT3'

The resulting heavy chain (see Fig. 4) when co-expressed with the humanised light chain (see Fig. 3) produces humanised anti-CD38,h3S.

(h) Eukaryotic expression of functional humanised antibody

To creat clonal cell lines for further characterisation, plasmids encoding the humanised h3S heavy chain and the chimaeric heavy chain were separately co-transfected with the humanised light chain into B11 CHO cells.

<u>Example 2</u>

<u>Biological activity</u>

(a) CD38 Binding Studies

(i) Effect of various heavy chain framework substitutions on relative binding affinity of anti-CD38 antibodies.

Binding was assessed by FACS staining of CD38 positive cells.

Heavy chains incorporating one or more of mouse framework residues were created as described above and combined with the humanised light chain to make antibodies which were assayed for binding to CD38, with the following results.

Construct	66-73	28/29	78	Binding
h1	-	-	-	-
h2	+	-	-	-
h3J	-	+	-	-
h3K	+	+	-	+
h3S	+	+	+	++

In this table, + denotes that the murine framework residue is incorporated into the humanised antibody at the indicated position, - denotes that the human residue remains.

<u>Discussion</u>

According to computer modelling studies the change of the 66-73 region back to mouse framework causes the humanised CDRH2 to adopt a similar conformation to that of the mouse antibody. However, as the construct h2 shows, this is insufficient to obtain binding. The model also suggests that in the mouse anti-CD38 antibody, positions 29 and 78 are occupied by small residues, whose side-

chains pack neatly together allowing CDRH1 to adopt the correct configuration for antigen binding. In the humanised constructs h1 and h2, the side chains are unable to pack together in this fashion, being much larger, and so distort CDRH1, preventing antigen binding. This aspect of the model is illustrated in Figures 5 and 6 (attached). Figure 5 shows the configuration of CDRH1 (dark tubes) in the murine anti-CD38. In Figure 6 showing the same region in a humanised construct with human residues at positions 29 and 78, the extra bulk of these side chains has clearly resulted in a distortion of the CDRH1 conformation.

Partial relief of this effect can be obtained by using the murine residue at position 29 and the human residue at position 78, though the resulting antibody shows markedly reduced function. Use of murine residues at both positions 29 and 78 restores activity, as evidenced by the data for the h3S construct.

(ii) Anti-CD38 heavy chain variable regions were fused to human γ1 constant region and coexpressed in CHO cells with humanised anti-CD38 light chain. CD38-binding activity is expressed normalised to the signal obtained using a saturating dose of hybrid antibody (mouse VH) in the same experiment.

Results are shown in Figure 7 where:

♦ Humanised antibody with murine residues at 28,29 and 78

▲ Humanised antibody with murine residues at 28,29 and 76

- ● Humanised antibody with murine residues at 28,29

- ■ Hybrid antibody

In addition to the above substitutions, all humanised heavy chains contained murine framework residues at positions 27, 30, 67, 68, 69, 70, 71, 73 and 94. These alone are insufficient to obtain detectable binding by FACS.

These results demonstrate the critical importance of the small residues at positions 29 and/or 78 in obtaining full humanised heavy chain activity. They also demonstrate the specific nature of the interaction, in that a murine residue at position 76 close to position 78 was unable to restor activity.

(b) Effect of various heavy chain framework substitutions on antibody-dependent cellular cytotoxicity mediated by CD38 antibodies.

Antibody-dependent cellular cytotoxicity is normally assessed by one of several label-release techniques, well-known in the literature. In one such assay, 10^4 target cells (Wien 133) were labelled with europium and then exposed to freshly prepared human peripheral blood lymphocytes in the presence of antibody as an effector:target ratio of 50:1. Lysis was estimated by detecting release of europium after 4 hours, and quantitated by reference to control reactions without antibody or peripheral blood lymphocytes or with detergent such as Triton-X100.

The effect of framework substitutions on the lytic potential of humanised anti-CD38 monoclonals was examined

in label-release assay. Wien 133 target cells were loaded with label (either 51Cr or Eu) and then exposed to freshly prepared human peripheral blood mononuclear cells in the presence of varying amounts of anti-CD38 antibody. Cytotoxicity is expressed as the proportion of total releasable label liberated by antibody treatment.

Results are shown in Figure 10 where:

- ▲ Humanised antibody with murine residues at 28,29 and 78
- ■ Humanised antibody with murine residues at 28,29 and 76
- ● Hybrid antibody

These results show that the combination of framework changes at positions 29 and 78 confer full activity on the humanised heavy chain for cytotoxic function. Although incorporation of a small murine residue at position 29 results in some binding activity (Figure 7), this is insufficient to achieve full effector function.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: The Wellcome Foundation Limited
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(D) STATE: Kent
(E) COUNTRY: United Kingdom
(F) POSTAL CODE (ZIP): BR3 3BS

(ii) TITLE OF INVENTION: ANTIBODIES

(iii) NUMBER OF SEQUENCES: 46

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 454 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..453

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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GTG CAC CCC TCA CAG AGC CTG TCC ATA ACC TGC ACA GTC TCT GGT TTC       144
Val His Pro Ser Gln Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe
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TCA TTA ACT AGT TAT GGT GTC CAC TGG GTT CGC CAG TCT CCA GGA AAG       192
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 65                  70                  75                  80

AAT GCA GCT TTC ATG TCC AGA CTG AAC ATC ACC AAG GAC AAC TCC AAG     288
Asn Ala Ala Phe Met Ser Arg Leu Asn Ile Thr Lys Asp Asn Ser Lys
                 85                  90                  95

CGC CAG GTT TTC TTT AAA ATG AAC AGT CTA CAA GCT GAT GAC ACT GCC     336
Arg Gln Val Phe Phe Lys Met Asn Ser Leu Gln Ala Asp Asp Thr Ala
            100                 105                 110

ATA TAC TAC TGT GCC AAA TCG ATG ATT ACG ACG GGC TTT GTT ATG GAC     384
Ile Tyr Tyr Cys Ala Lys Ser Met Ile Thr Thr Gly Phe Val Met Asp
        115                 120                 125

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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 151 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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35 40 45

Ser Leu Thr Ser Tyr Gly Val His Trp Val Arg Gln Ser Pro Gly Lys
50 55 60

Gly Leu Glu Trp Leu Gly Val Met Trp Arg Gly Gly Ser Thr Asp Tyr
65 70 75 80

Asn Ala Ala Phe Met Ser Arg Leu Asn Ile Thr Lys Asp Asn Ser Lys
85 90 95

Arg Gln Val Phe Phe Lys Met Asn Ser Leu Gln Ala Asp Asp Thr Ala
100 105 110

Ile Tyr Tyr Cys Ala Lys Ser Met Ile Thr Thr Gly Phe Val Met Asp
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(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 454 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

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TATTGGACGT GTCAGAGACC AAAGAGTAAT TGATCAATAC CACAGGTGAC CCAAGCGGTC 180

AGAGGTCCTT TCCCAGACCT CACCGACCCT CACTACACCT CTCCACCTTC GTGTCTGATG 240

TTACGTCGAA AGTACAGGTC TGACTTGTAG TGGTTCCTGT TGAGGTTCGC GGTCCAAAAG 300

AAATTTTACT TGTCAGATGT TCGACTACTG TGACGGTATA TGATGACACG GTTTAGCTAC 360

TAATGCTGCC CGAAACAATA CCTGAGGACC CCAGTTCCTT GGAGTCAGTG GCAGAGGAGT 420

CGGTTTTGCT GTGGGGGTAG ACAGATAGGT GACC 454

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 364 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION:1..363

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

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Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Glu Asp Ile Tyr Asn Arg
        170                 175                 180

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Leu Thr Trp Tyr Gln Gln Lys Pro Gly Asn Ala Pro Arg Leu Leu Ile
    185                 190                 195

TCT GGT GCA ACC AGT TTG GAA ACT GGG GTT CCT TCA AGA TTC AGT GGC     192
Ser Gly Ala Thr Ser Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
200                 205                 210                 215

AGT GGA TCT GGA AAG GAT TAC ACT CTC AGC ATT ACC AGT CTT CAG ACT     240
Ser Gly Ser Gly Lys Asp Tyr Thr Leu Ser Ile Thr Ser Leu Gln Thr
                220                 225                 230

GAA GAT GTT GCT ACC TAT TAC TGT CAA CAG TAT TGG AGT AAT CCG TAC     288
Glu Asp Val Ala Thr Tyr Tyr Cys Gln Gln Tyr Trp Ser Asn Pro Tyr
            235                 240                 245
```

ACG TTC GGA GGG GGG ACC AAG CTG GAA ATA AGA CGG GCT GAT GCT GCA 336
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Arg Arg Ala Asp Ala Ala
250 255 260

CCA ACT GTA TCC ATC TTC CCA CCA TCC A 364
Pro Thr Val Ser Ile Phe Pro Pro Ser
265 270

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 121 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Phe Ser Val Ser Leu Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Glu Asp Ile Tyr Asn Arg
20 25 30

Leu Thr Trp Tyr Gln Gln Lys Pro Gly Asn Ala Pro Arg Leu Leu Ile
35 40 45

Ser Gly Ala Thr Ser Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Lys Asp Tyr Thr Leu Ser Ile Thr Ser Leu Gln Thr
65 70 75 80

Glu Asp Val Ala Thr Tyr Tyr Cys Gln Gln Tyr Trp Ser Asn Pro Tyr

85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Arg Arg Ala Asp Ala Ala
100 105 110

Pro Thr Val Ser Ile Phe Pro Pro Ser
115 120

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 364 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

CTGTAAGTCG ACTGGGTCAG AGGTAGGAGG AAAAGACAAA GAGATCCTCT GTCTCAGTGG 60

TAATGAACGT TCCGTTCACT CCTGTATATA TTAGCCAATT GGACCATAGT CGTCTTTGGT 120

CCTTTACGAG GATCCGAGAA TTATAGACCA CGTTGGTCAA ACCTTTGACC CCAAGGAAGT 180

TCTAAGTCAC CGTCACCTAG ACCTTTCCTA ATGTGAGAGT CGTAATGGTC AGAAGTCTGA 240

CTTCTACAAC GATGGATAAT GACAGTTGTC ATAACCTCAT TAGGCATGTG CAAGCCTCCC 300

CCCTGGTTCG ACCTTTATTC TGCCCGACTA CGACGTGGTT GACATAGGTA GAAGGGTGGT 360

AGGT 364

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 746 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:3..737

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```
AA GCT TCT CTA CAG TTA CTG AGC ACA CAG GAC CTC ACC ATG GGA TGG      47
   Ala Ser Leu Gln Leu Leu Ser Thr Gln Asp Leu Thr Met Gly Trp
               125                 130                 135

AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC TCC     95
Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly Val His Ser
            140                 145                 150

GAC ATC CAG ATG ACC CAG AGC CCA AGC AGC CTG AGC GCC AGC GTG GGT    143
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
        155                 160                 165

GAC AGA GTG ACC ATC ACC TGT AAG GCA AGT GAG GAC ATA TAT AAT CGG    191
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Glu Asp Ile Tyr Asn Arg
    170                 175                 180
```

TTA ACC TGG TAC CAG CAG AAG CCA GGT AAG GCT CCA AAG CTG CTG ATC 239
Leu Thr Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
185 190 195 200

TCT GGT GCA ACC AGT TTG GAA ACT GGT GTG CCA AGC AGA TTC AGC GGT 287
Ser Gly Ala Thr Ser Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
205 210 215

AGC GGT AGC GGT ACC GAC TTC ACC TTC ACC ATC AGC AGC CTC CAG CCA 335
Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro
220 225 230

GAG GAC ATC GCC ACC TAC TAC TGC CAA CAG TAT TGG AGT AAT CCG TAC 383
Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Trp Ser Asn Pro Tyr
235 240 245

ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA CGA ACT GTG GCT GCA 431
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
250 255 260

CCA TCT GTC TTC ATC TTC CCG CCA TCT GAT GAG CAG TTG AAA TCT GGA 479
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
265 270 275 280

ACT GCC TCT GTT GTG TGC CTG CTG AAT AAC TTC TAT CCC AGA GAG GCC 527
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
285 290 295

AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG GGT AAC TCC CAG 575
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
300 305 310

GAG AGT GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC AGC 623
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
315 320 325

AGC ACC CTG ACG CTG AGC AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC 671
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
330 335 340

GCC TGC GAA GTC ACC CAT CAG GGC CTG AGC TCG CCC GTC ACA AAG AGC 719
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
345 350 355 360

TTC AAC AGG GGA GAG TGT TAGAAGCTT 746
Phe Asn Arg Gly Glu Cys
365

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 245 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Ala Ser Leu Gln Leu Leu Ser Thr Gln Asp Leu Thr Met Gly Trp Ser
1 5 10 15

Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly Val His Ser Asp
20 25 30

Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp
35 40 45

Arg Val Thr Ile Thr Cys Lys Ala Ser Glu Asp Ile Tyr Asn Arg Leu
50 55 60

Thr Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Ser
65 70 75 80

Gly Ala Thr Ser Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly Ser
85 90 95

Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
100 105 110

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Trp Ser Asn Pro Tyr Thr
115 120 125

Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
130 135 140

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
145 150 155 160

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
165 170 175

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
180 185 190

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
195 200 205

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
210 215 220

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
225 230 235 240

Asn Arg Gly Glu Cys
245

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 746 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

TTCGAAGAGA TGTCAATGAC TCGTGTGTCC TGGAGTGGTA CCCTACCTCG ACATAGTAGG 60

AGAAGAACCA TCGTTGTCGA TGTCCACAGG TGAGGCTGTA GGTCTACTGG GTCTCGGGTT 120

CGTCGGACTC GCGGTCGCAC CCACTGTCTC ACTGGTAGTG GACATTCCGT TCACTCCTGT 180

ATATATTAGC CAATTGGACC ATGGTCGTCT TCGGTCCATT CCGAGGTTTC GACGACTAGA 240

GACCACGTTG GTCAAACCTT TGACCACACG GTTCGTCTAA GTCGCCATCG CCATCGCCAT 300

GGCTGAAGTG GAAGTGGTAG TCGTCGGAGG TCGGTCTCCT GTAGCGGTGG ATGATGACGG 360

TTGTCATAAC CTCATTAGGC ATGTGCAAGC CGGTTCCCTG GTTCCACCTT TAGTTTGCTT 420

GACACCGACG TGGTAGACAG AAGTAGAAGG GCGGTAGACT ACTCGTCAAC TTTAGACCTT 480

GACGGAGACA ACACACGGAC GACTTATTGA AGATAGGGTC TCTCCGGTTT CATGTCACCT 540

TCCACCTATT GCGGGAGGTT AGCCCATTGA GGGTCCTCTC ACAGTGTCTC GTCCTGTCGT 600

TCCTGTCGTG GATGTCGGAG TCGTCGTGGG ACTGCGACTC GTTTCGTCTG ATGCTCTTTG 660

TGTTTCAGAT GCGGACGCTT CAGTGGGTAG TCCCGGACTC GAGCGGGCAG TGTTTCTCGA 720

AGTTGTCCCC TCTCACAATC TTCGAA 746

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 436 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:3..14

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:18..434

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

```
AA GCT TTA CAG TTA CNC AGC ACA CAG GAC CTC ACC ATG GGA TGG AGC      47
   Ala Leu Gln Leu     Ser Thr Gln Asp Leu Thr Met Gly Trp Ser
                         1               5                  10

TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC TCC CAG     95
Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly Val His Ser Gln
                 15                  20                  25
```

GTC CAA CTG CAG GAG AGC GGT CCA GGT CTT GTG AGA CCT AGC CAG ACC 143
Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln Thr
30 35 40

CTG AGC CTG ACC TGC ACC GTG TCT GGC TTT TCG TTA ACC AGT TAT GGT 191
Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ser Tyr Gly
45 50 55

GTC CAC TGG GTG AGA CAG CCA CCT GGA CGA GGT CTT GAG TGG ATT GGA 239
Val His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile Gly
60 65 70

GTG ATG TGG AGA GGT GGA AGC ACA GAC TAC AAT GCA GCT TTC ATG TCC 287
Val Met Trp Arg Gly Gly Ser Thr Asp Tyr Asn Ala Ala Phe Met Ser
75 80 85 90

AGA CTG AAC ATC ACC AAG GAC AAC AGC AAG AAC CAG GTG AGC TTA AGA 335
Arg Leu Asn Ile Thr Lys Asp Asn Ser Lys Asn Gln Val Ser Leu Arg
95 100 105

CTC AGC AGC GTG ACA GCC GCC GAC ACC GCG GTC TAT TAT TGT GCA AAA 383
Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Lys
110 115 120

TCC ATG ATA ACG ACC GGC TTC GTG ATG GAC TCC TGG GGT CAA GGC TCA 431
Ser Met Ile Thr Thr Gly Phe Val Met Asp Ser Trp Gly Gln Gly Ser
125 130 135

CTA GT 436
Leu

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```
Ala Leu Gln Leu
  1
```

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 139 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

```
Ser Thr Gln Asp Leu Thr Met Gly Trp Ser Cys Ile Ile Leu Phe Leu
  1               5                  10                  15

Val Ala Thr Ala Thr Gly Val His Ser Gln Val Gln Leu Gln Glu Ser
             20                  25                  30

Gly Pro Gly Leu Val Arg Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr
         35                  40                  45

Val Ser Gly Phe Ser Leu Thr Ser Tyr Gly Val His Trp Val Arg Gln
     50                  55                  60

Pro Pro Gly Arg Gly Leu Glu Trp Ile Gly Val Met Trp Arg Gly Gly
```

65 70 75 80

Ser Thr Asp Tyr Asn Ala Ala Phe Met Ser Arg Leu Asn Ile Thr Lys
85 90 95

Asp Asn Ser Lys Asn Gln Val Ser Leu Arg Leu Ser Ser Val Thr Ala
100 105 110

Ala Asp Thr Ala Val Tyr Tyr Cys Ala Lys Ser Met Ile Thr Thr Gly
115 120 125

Phe Val Met Asp Ser Trp Gly Gln Gly Ser Leu
130 135

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 436 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

TTCGAAATGT CAATGNGTCG TGTGTCCTGG AGTGGTACCC TACCTCGACA TAGTAGGAGA 60

AGAACCATCG TTGTCGATGT CCACAGGTGA GGGTCCAGGT TGACGTCCTC TCGCCAGGTC 120

CAGAACACTC TGGATCGGTC TGGGACTCGG ACTGGACGTG GCACAGACCG AAAAGCAATT 180

GGTCAATACC ACAGGTGACC CACTCTGTCG GTGGACCTGC TCCAGAACTC ACCTAACCTC 240

ACTACACCTC TCCACCTTCG TGTCTGATGT TACGTCGAAA GTACAGGTCT GACTTGTAGT 300

GGTTCCTGTT GTCGTTCTTG GTCCACTCGA ATTCTGAGTC GTCGCACTGT CGGCGGCTGT 360

GGCGCCAGAT AATAACACGT TTTAGGTACT ATTGCTGGCC GAAGCACTAC CTGAGGACCC 420

CAGTTCCGAG TGATCA 436

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GACATTCAGC TGACCCAGTC TCCA 24

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

GATCAAGCTT GACATTCAGC TGACCCAGTC TCCA 34

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

ACTAGTCGAC CATCCTCCTT TTCTGTTTCT CTAGGAG 37

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GATCAAGCTT CTCTACAGTT ACTGAGCACA 30

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

CCGATTATAT ATGTCCTCAC TTGCCTTACA GGTGATGGTC AC 42

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

AGTGAGGACA TATATAATCG GTTAACCTGG TACCAGCAGA AG 42

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

AGTTTCCAAA CTGGTTGCAC CAGAGATCAG CAGCTTTGG 39

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

GGTGCAACCA GTTTGGAAAC TGGTGTGCCA AGCAGA 36

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

GTACGGATTA CTCCAATACT GTTGGCAGTA GTAGGTGGC 39

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

CAGTATTGGA GTAATCCGTA CACGTTCGGC CAAGGGACC 39

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

GATCAAGCTT CTAACACTCT CCCCTGTTGA 30

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

GATCAAGCTT TACAGTTACT CAGCACACAG 30

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

GTGGACACCA TAACTGGTGA AGGTGAAGCC 30

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

AGTTATGGTG TCCACTGGGT GAGACAGCCA 30

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 47 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

TTGTAGTCTG TGCTTCCACC TCTCCACATC ACTCCAATCC ACTCAAG 47

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

GGAGTCCATC ACGAAGCCGG TCGTTATCAT GGATTTTGCA CAATAATAGA C 51

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

AAATCCATGA TAACGACCGG CTTCGTGATG GACTCCTGGG GTCAAGGCTC ACTAGTCACA 60

GTCTCCTCAG CC 72

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

TAGAGTCCTG AGGGAATTCG GACAGCCGGG AAGGTG 36

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

GTTGTCCTTG GTGATGTTCA GTCTGGACAT GAAAGCTGC 39

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

CTGAACATCA CCAAGGACAA CAGCAAGAAC CAGTTCAGC 39

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

ACTGGTTAAC GAAAAGCCAG ACACGGTGCA GGTCAG 36

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

GGCTTTTCGT TAACCAGTTA TGGTGTCCAC TGGGTG 36

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

AAATTGCCGT TTCGAAGTGT CTACCAGCAT TGTCAC 36

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

AAATTGCCGT TTCGAATTGT CCTTGGTGAT GTTCAG 36

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

TTCGAAACGG CAATTTAGCT TGAGACTCAG CAGC 34

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 47 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

GAAGCACAGA CTACAATGCA GCTTTCATGT CCAGAGTGAC AATGCTG 47

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

GATCAAGCTT TACAGTTACT CAGCACAG 28

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

ACTGGTTAAC GAAAAGCCAG ACACGGTGCA GGTCAG 36

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

GGCTTTTCGT TAACCAGTTA TGGTGTCCAC TGGGTG 36

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

TAGAGTCCTG AGGGAATTCG GACAGCCGGG AAGGTG 36

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

AACCAGGTGA GCTTAAGACT CAGCAGCGTG ACA 33

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

TCTTAAGCTC ACCTGGTTCT TGCTGTTGTC CTT 33

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

Ser Tyr Gly Val His
1 5

CLAIMS:

1. A monoclonal antibody having donor CDRs of foreign origin and a recipient framework region having a sequence of human or primate origin, wherein the original amino acid residue in position 29 or 78 of the sequence of the recipient framework region of the heavy chain is replaced by a replacement amino acid residue that is the same or similar to that in the corresponding position of the sequence of the corresponding framework region of the heavy chain of the antibody from which the CDRs are derived.

2. A monoclonal antibody according to claim 1, wherein the original amino acid residues in both positions 29 and 78 of the sequence of the recipient framework region of the heavy chain are replaced by replacement amino acids that are the same or similar to the amino acids in the corresponding positions of the corresponding framework region of the antibody from which the CDRs are derived.

3. A monoclonal antibody according to claim 1 or 2, wherein one or both of the original amino acid residues of the recipient framework region are replaced by a replacement amino acid residues of similar size, hydrophobicity and charge to the amino acids in the corresponding positions of the corresponding framework region of the antibody from which the CDRs are derived.

4. A monoclonal antibody according to any of the preceding claims, wherein the original amino acid residues of the recipient framework region are the same or different and are tyrosine, histidine, tryptophan or 2-phenyl-alanine.

5. A monoclonal antibody according to claim 4, wherein the replacement amino acid residues are the same or different and are selected from glycine, alanine, valine, serine or leucine.

6. A monoclonal antibody according to any of the preceding claims wherein the recipient framework region is from a heavy chain selected from LES-C, T52, Ab44, HIGI and NEW.

7. A monoclonal antibody according to any of the preceding claims, wherein the CDRs are of rat, mouse rabbit, or hamster origin.

8. A monoclonal antibody according to any of the preceding claims, wherein the heavy chain of the antibody from which the CDRs are derived is a murine heavy chain in Kabat groups IB and IIC.

9. A monoclonal antibody according to any of the preceding claims wherein the antibody binds to CD38.

10. A monoclonal antibody according to claim 9 having a nucleotide sequence as shown in figures 3, 3a and 4.

11. A monoclonal antibody according to any of the preceding claims, wherein the donor CDR is CDRHI.

12. A monoclonal antibody according to claim 11, wherein CDRHI has a sequence of SYGVH.

13. A method of producing an antibody according to any of the above claims comprising the steps of:

(i) obtaining the sequence of a donor heavy chain;

(ii) selecting a recipient human or primate framework by best-fit homology method;

(iii) replacing the amino acid residue in position 29 or 78 of the sequence of the recipient framework region of the heavy chain by an amino acid that is the same or similar to that in the corresponding position of the sequence of the corresponding framework region of the antibody from which the CDRs are derived;

(iv) grafting donor CDRs into the recipient human framework.

14. Use of an antibody according to any of the preceding claims for the treatment of cancer and autoimmune diseases.

15. Use of an antibody according to claim 9 or 10 for treatment of multiple myeloma, lymphoma and autoimmune diseases such as rheumatoid arthritis.

16. Use of an antibody according to any of claims 1 to 12 for the manufacture of a medicament for the treatment of cancer or an autoimmune disease.

17. Use of an antibody according to any of claims 1 to 12 for the manufacture of a medicament for the treatment of multiple myeloma, lymphoma, or rheumatoid arthritis.

18. A pharmaceutical composition comprising an antibody according to any of claims 1 to 12 and a physiologically acceptable diluent or carrier.

```
        GGTCGACTGGCTGTGTTAGCGCTGCTCTTCTGCCTGGTgACATTCCCAAGCTGTGTCCTG
     1  ---------+---------+---------+---------+---------+---------+ 60
        CCAGCTGACCGACACAATCGCGACGAGAAGACGGACCAcTGTAAGGGTTCGACACAGGAC

a       G  R  L  A  V  L  A  L  L  F  C  L  V  T  F  P  S  C  V  L  -

        TCCCAGGTGCAGCTGAAGCAGTCAGgaCCTGGCCTAGTGCACCCCTCACAGAGCCTGTCC
    61  ---------+---------+---------+---------+---------+---------+ 120
        AGGGTCCACGTCGACTTCGTCAGTCctGGACCGGATCACGTGGGGAGTGTCTCGGACAGG

a       S  Q  V  Q  L  K  Q  S  G  P  G  L  V  H  P  S  Q  S  L  S  -

        ATAACCTGCACAGTCTCTGGTTTCTCATTAACTAGTTATGGTGTCCACTGGGTTCGCCAG
   121  ---------+---------+---------+---------+---------+---------+ 180
        TATTGGACGTGTCAGAGACCAAAGAGTAATTGATCAATACCACAGGTGACCCAAGCGGTC

a       I  T  C  T  V  S  G  F  S  L  T  S  Y  G  V  H  W  V  R  Q  -

        TCTCCAGGAAAGGGTCTGGAGTGGCTGGGAGTGATGTGGAGAGGTGGAAGCACAGACTAC
   181  ---------+---------+---------+---------+---------+---------+ 240
        AGAGGTCCTTTCCCAGACCTCACCGACCCTCACTACACCTCTCCACCTTCGTGTCTGATG

a       S  P  G  K  G  L  E  W  L  G  V  M  W  R  G  G  S  T  D  Y  -

        aATgCAGCTTTCAtGTCCAGACTGAACAtcACCAAGGACAACTCCAAGCGCCAGGTTTTC
   241  ---------+---------+---------+---------+---------+---------+ 300
        tTAcGTCGAAAGTACAGGTCTGACTTGTagTGGTTCCTGTTGAGGTTCGCGGTCCAAAAG

a       N  A  A  F  M  S  R  L  N  I  T  K  D  N  S  K  R  Q  V  F  -

        TTTAAAATGAACAGTCTACAAGCTGATGACACTGCCATATACTACTGTGCCAAATCGATG
   301  ---------+---------+---------+---------+---------+---------+ 360
        AAATTTTACTTGTCAGATGTTCGACTACTGTGACGGTATATGATGACACGGTTTAGCTAC

a       F  K  M  N  S  L  Q  A  D  D  T  A  I  Y  Y  C  A  K  S  M  -

        ATTACGACGGGCTTTGTTATGGACTCCTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCA
   361  ---------+---------+---------+---------+---------+---------+ 420
        TAATGCTGCCCGAAACAATACCTGAGGACCCCAGTTCCTTGGAGTCAGTGGCAGAGGAGT

a       I  T  T  G  F  V  M  D  S  W  G  Q  G  T  S  V  T  V  S  S  -

        GCCAAAACGACACCCCCATCTGTCTATCCACTGG
   421  ---------+---------+---------+---- 454
        CGGTTTTGCTGTGGGGGTAGACAGATAGGTGACC

a       A  K  T  T  P  P  S  V  Y  P  L  -
```

Fig. 1

```
      GACATTCAGCTGACCCAGTCTCCATCCTCCTTTTCTGTTTCTCTAGGAGACAGAGTCACC
    1 ---------+---------+---------+---------+---------+---------+ 60
      CTGTAAGTCGACTGGGTCAGAGGTAGGAGGAAAAGACAAAGAGATCCTCTGTCTCAGTGG

a     D  I  Q  L  T  Q  S  P  S  S  F  S  V  S  L  G  D  R  V  T  -

      ATTACTTGCAAGGCAAGTGAGGACATATATAATCGGTTAACCTGGTATCAGCAGAAACCA
   61 ---------+---------+---------+---------+---------+---------+ 120
      TAATGAACGTTCCGTTCACTCCTGTATATATTAGCCAATTGGACCATAGTCGTCTTTGGT

a     I  T  C  K  A  S  E  D  I  Y  N  R  L  T  W  Y  Q  Q  K  P  -

      GGAAATGCTCCTAGGCTCTTAATATCTGGTGCAACCAGTTTGGAAACTGGGGTTCCTTCA
  121 ---------+---------+---------+---------+---------+---------+ 180
      CCTTTACGAGGATCCGAGAATTATAGACCACGTTGGTCAAACCTTTGACCCCAAGGAAGT

a     G  N  A  P  R  L  L  I  S  G  A  T  S  L  E  T  G  V  P  S  -

      AGATTCAGTGGCAGTGGATCTGGAAAGGATTACACTCTCAGCATTACCAGTCTTCAGACT
  181 ---------+---------+---------+---------+---------+---------+ 240
      TCTAAGTCACCGTCACCTAGACCTTTCCTAATGTGAGAGTCGTAATGGTCAGAAGTCTGA

a     R  F  S  G  S  G  S  G  K  D  Y  T  L  S  I  T  S  L  Q  T  -

      GAAGATGTTGCTACCTATTACTGTCAACAGTATTGGAGTAATCCGTACACGTTCGGAGGG
  241 ---------+---------+---------+---------+---------+---------+ 300
      CTTCTACAACGATGGATAATGACAGTTGTCATAACCTCATTAGGCATGTGCAAGCCTCCC

a     E  D  V  A  T  Y  Y  C  Q  Q  Y  W  S  N  P  Y  T  F  G  G  -

      GGGACCAAGCTGGAAATAAGACGGGCTGATGCTGCACCAACTGTATCCATCTTCCCACCA
  301 ---------+---------+---------+---------+---------+---------+ 360
      CCCTGGTTCGACCTTTATTCTGCCCGACTACGACGTGGTTGACATAGGTAGAAGGGTGGT

a     G  T  K  L  E  I  R  R  A  D  A  A  P  T  V  S  I  F  P  P  -

      TCCA
  361 ---- 364
      AGGT

a     S  -
```

Fig. 2

```
      AAGCTTCTCTACAGTTACTGAGCACACAGGACCTCACCATGGGATGGAGCTGTATCATCC
   1  ---------+---------+---------+---------+---------+---------+ 60
      TTCGAAGAGATGTCAATGACTCGTGTGTCCTGGAGTGGTACCCTACCTCGACATAGTAGG

c      A  S  L  Q  L  L  S  T  Q  D  L  T  M  G  W  S  C  I  I  L -

      TCTTCTTGGTAGCAACAGCTACAGGTGTCCACTCCGACATCCAGATGACCCAGAGCCCAA
  61  ---------+---------+---------+---------+---------+---------+ 120
      AGAAGAACCATCGTTGTCGATGTCCACAGGTGAGGCTGTAGGTCTACTGGGTCTCGGGTT

c      F  L  V  A  T  A  T  G  V  H  S  D  I  Q  M  T  Q  S  P  S -

      GCAGCCTGAGCGCCAGCGTGGGTGACAGAGTGACCATCACCTGTAAGGCAAGTGAGGACA
 121  ---------+---------+---------+---------+---------+---------+ 180
      CGTCGGACTCGCGGTCGCACCCACTGTCTCACTGGTAGTGGACATTCCGTTCACTCCTGT

c      S  L  S  A  S  V  G  D  R  V  T  I  T  C  K  A  S  E  D  I -

      TATATAATCGGTTAACCTGGTACCAGCAGAAGCCAGGTAAGGCTCCAAAGCTGCTGATCT
 181  ---------+---------+---------+---------+---------+---------+ 240
      ATATATTAGCCAATTGGACCATGGTCGTCTTCGGTCCATTCCGAGGTTTCGACGACTAGA

c      Y  N  R  L  T  W  Y  Q  Q  K  P  G  K  A  P  K  L  L  I  S -

      CTGGTGCAACCAGTTTGGAAACTGGTGTGCCAAGCAGATTCAGCGGTAGCGGTAGCGGTA
 241  ---------+---------+---------+---------+---------+---------+ 300
      GACCACGTTGGTCAAACCTTTGACCACACGGTTCGTCTAAGTCGCCATCGCCATCGCCAT

c      G  A  T  S  L  E  T  G  V  P  S  R  F  S  G  S  G  S  G  T -

      CCGACTTCACCTTCACCATCAGCAGCCTCCAGCCAGAGGACATCGCCACCTACTACTGCC
 301  ---------+---------+---------+---------+---------+---------+ 360
      GGCTGAAGTGGAAGTGGTAGTCGTCGGAGGTCGGTCTCCTGTAGCGGTGGATGATGACGG

c      D  F  T  F  T  I  S  S  L  Q  P  E  D  I  A  T  Y  Y  C  Q -

      AACAGTATTGGAGTAATCCGTACACGTTCGGCCAAGGGACCAAGGTGGAAATCAAACGAA
 361  ---------+---------+---------+---------+---------+---------+ 420
      TTGTCATAACCTCATTAGGCATGTGCAAGCCGGTTCCCTGGTTCCACCTTTAGTTTGCTT

c      Q  Y  W  S  N  P  Y  T  F  G  Q  G  T  K  V  E  I  K  R  T -

      CTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAA
 421  ---------+---------+---------+---------+---------+---------+ 480
      GACACCGACGTGGTAGACAGAAGTAGAAGGGCGGTAGACTACTCGTCAACTTTAGACCTT

c      V  A  A  P  S  V  F  I  F  P  P  S  D  E  Q  L  K  S  G  T -

      CTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGA
 481  ---------+---------+---------+---------+---------+---------+ 540
      GACGGAGACAACACACGGACGACTTATTGAAGATAGGGTCTCTCCGGTTTCATGTCACCT

c      A  S  V  V  C  L  L  N  N  F  Y  P  R  E  A  K  V  Q  W  K -

      AGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCA
 541  ---------+---------+---------+---------+---------+---------+ 600
      TCCACCTATTGCGGGAGGTTAGCCCATTGAGGGTCCTCTCACAGTGTCTCGTCCTGTCGT

c      V  D  N  A  L  Q  S  G  N  S  Q  E  S  V  T  E  Q  D  S  K -

      AGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAAC
 601  ---------+---------+---------+---------+---------+---------+ 660
      TCCTGTCGTGGATGTCGGAGTCGTCGTGGGACTGCGACTCGTTTCGTCTGATGCTCTTTG
```

Fig. 3

```
c           D  S  T  Y  S  L  S  S  T  L  T  L  S  K  A  D  Y  E  K  H -

       ACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCT
  661 ---------+---------+---------+---------+---------+---------+ 720
       TGTTTCAGATGCGGACGCTTCAGTGGGTAGTCCCGGACTCGAGCGGGCAGTGTTTCTCGA

c           K  V  Y  A  C  E  V  T  H  Q  G  L  S  S  P  V  T  K  S  F -

       TCAACAGGGGAGAGTGTTAGAAGCTT
  721 ---------+---------+------ 660
       AGTTGTCCCCTCTCACAATCTTCGAA

c           N  R  G  E  C  *  K  L   -
```

Fig.3a

```
     AAGCTTTACAGTTACXCAGCACACAGGACCTCACCATGGGATGGAGCTGTATCATCCTCT
   1 ---------+---------+---------+---------+---------+---------+ 60
     TTCGAAATGTCAATGXGTCGTGTGTCCTGGAGTGGTACCCTACCTCGACATAGTAGGAGA

c      A  L  Q  L  ?  S  T  Q  D  L  T  M  G  W  S  C  I  I  L  F -

     TCTTGGTAGCAACAGCTACAGGTGTCCACTCCCAGGTCCAACTGCAGGAGAGCGGTCCAG
  61 ---------+---------+---------+---------+---------+---------+ 120
     AGAACCATCGTTGTCGATGTCCACAGGTGAGGGTCCAGGTTGACGTCCTCTCGCCAGGTC

c      L  V  A  T  A  T  G  V  H  S  Q  V  Q  L  Q  E  S  G  P  G -

     GTCTTGTGAGACCTAGCCAGACCCTGAGCCTGACCTGCACCGTGTCTGGCTTTTCGTTAA
 121 ---------+---------+---------+---------+---------+---------+ 180
     CAGAACACTCTGGATCGGTCTGGGACTCGGACTGGACGTGGCACAGACCGAAAAGCAATT

c      L  V  R  P  S  Q  T  L  S  L  T  C  T  V  S  G  F  S  L  T -

     CCAGTTATGGTGTCCACTGGGTGAGACAGCCACCTGGACGAGGTCTTGAGTGGATTGGAG
 181 ---------+---------+---------+---------+---------+---------+ 240
     GGTCAATACCACAGGTGACCCACTCTGTCGGTGGACCTGCTCCAGAACTCACCTAACCTC

c      S  Y  G  V  H  W  V  R  Q  P  P  G  R  G  L  E  W  I  G  V -

     TGATGTGGAGAGGTGGAAGCACAGACTACAATGCAGCTTTCATGTCCAGACTGAACATCA
 241 ---------+---------+---------+---------+---------+---------+ 300
     ACTACACCTCTCCACCTTCGTGTCTGATGTTACGTCGAAAGTACAGGTCTGACTTGTAGT

c      M  W  R  G  G  S  T  D  Y  N  A  A  F  M  S  R  L  N  I  T -

     CCAAGGACAACAGCAAGAACCAGGTGAGCTTAAGACTCAGCAGCGTGACAGCCGCCGACA
 301 ---------+---------+---------+---------+---------+---------+ 360
     GGTTCCTGTTGTCGTTCTTGGTCCACTCGAATTCTGAGTCGTCGCACTGTCGGCGGCTGT

c      K  D  N  S  K  N  Q  V  S  L  R  L  S  S  V  T  A  A  D  T -

     CCGCGGTCTATTATTGTGCAAAATCCATGATAACGACCGGCTTCGTGATGGACTCCTGGG
 361 ---------+---------+---------+---------+---------+---------+ 420
     GGCGCCAGATAATAACACGTTTTAGGTACTATTGCTGGCCGAAGCACTACCTGAGGACCC

c      A  V  Y  Y  C  A  K  S  M  I  T  T  G  F  V  M  D  S  W  G -

     GTCAAGGCTCACTAGT
 421 ---------+------ 436
     CAGTTCCGAGTGATCA

c      Q  G  S  L     -
```

Fig. 4

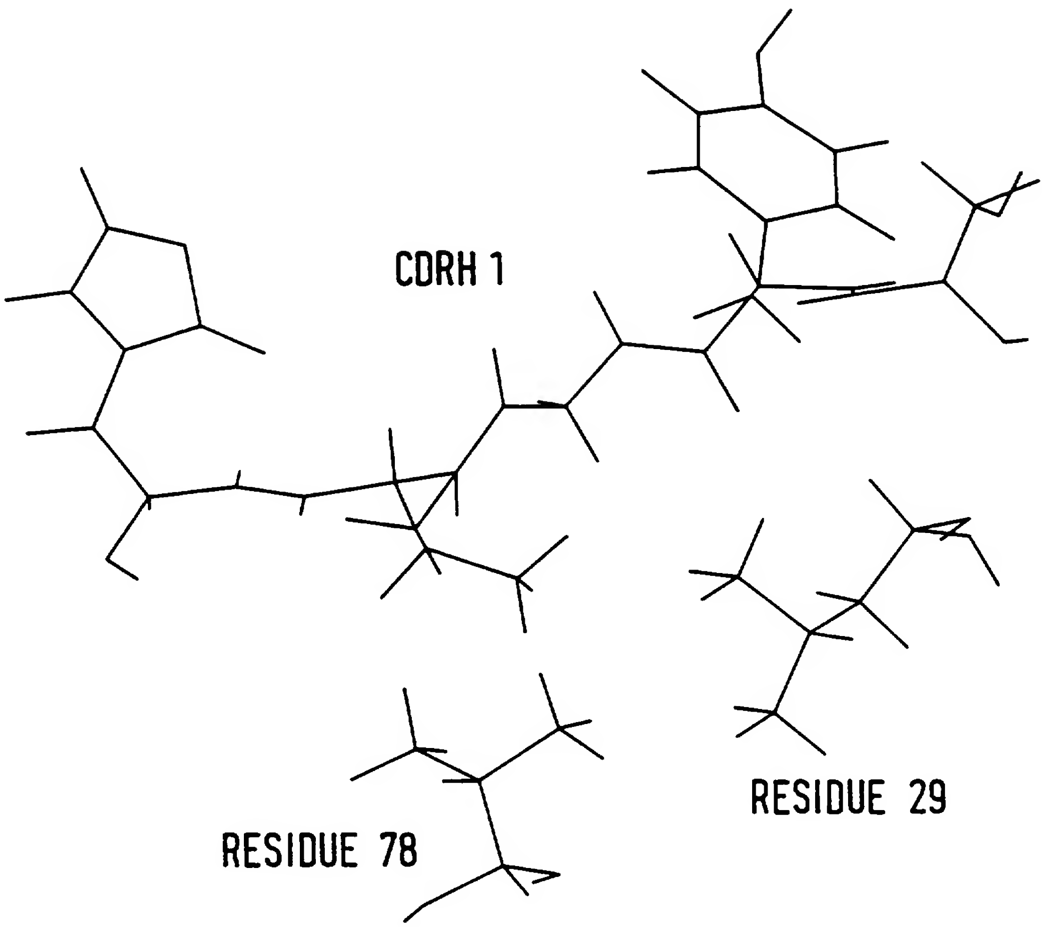

Fig. 5

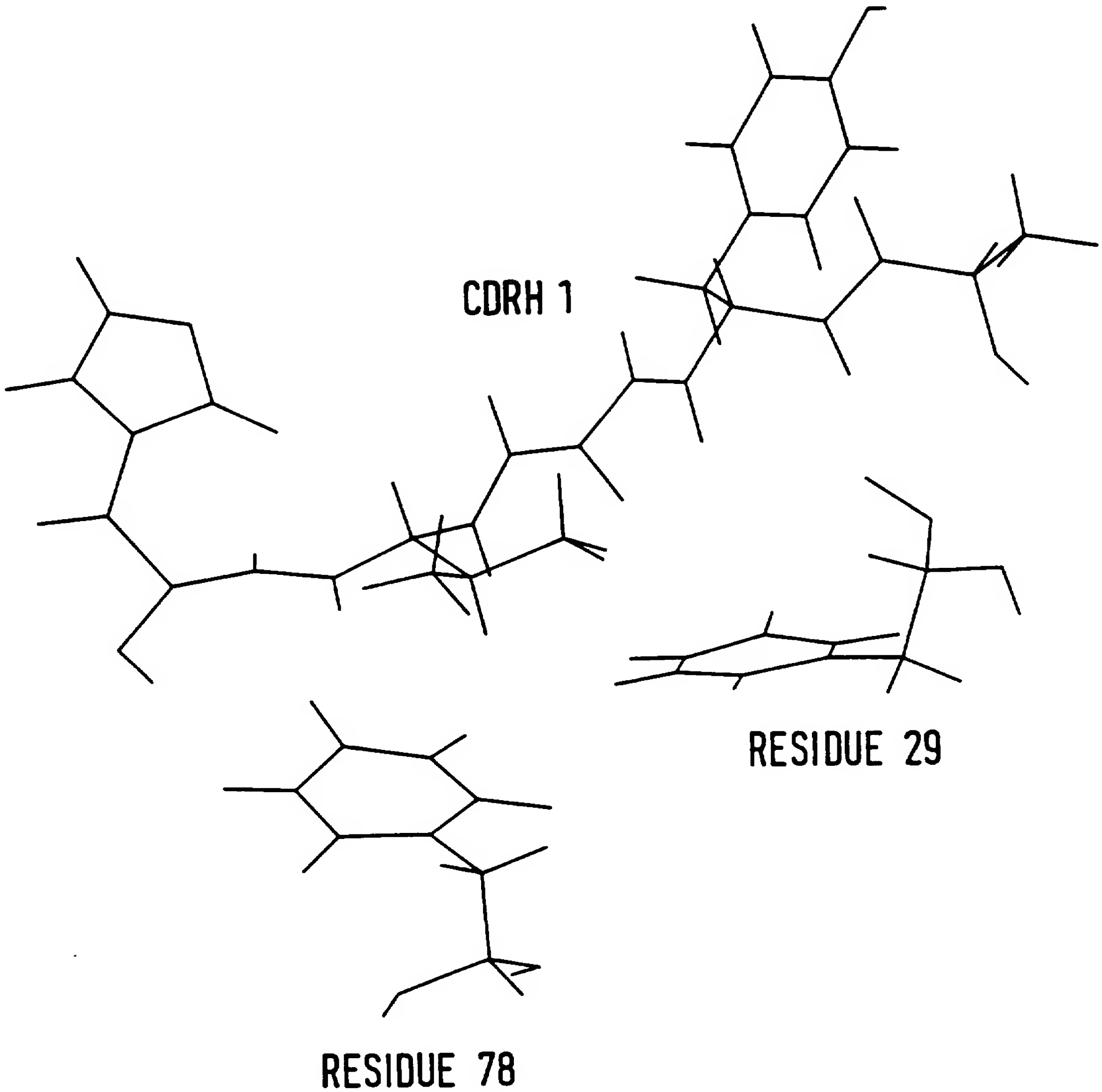

Fig. 6

EFFECT OF VARIOUS HEAVY CHAIN FRAMEWORK SUBSTITUTIONS ON RELATIVE BINDING AFFINITY OF ANT-CD38 ANTIBODIES

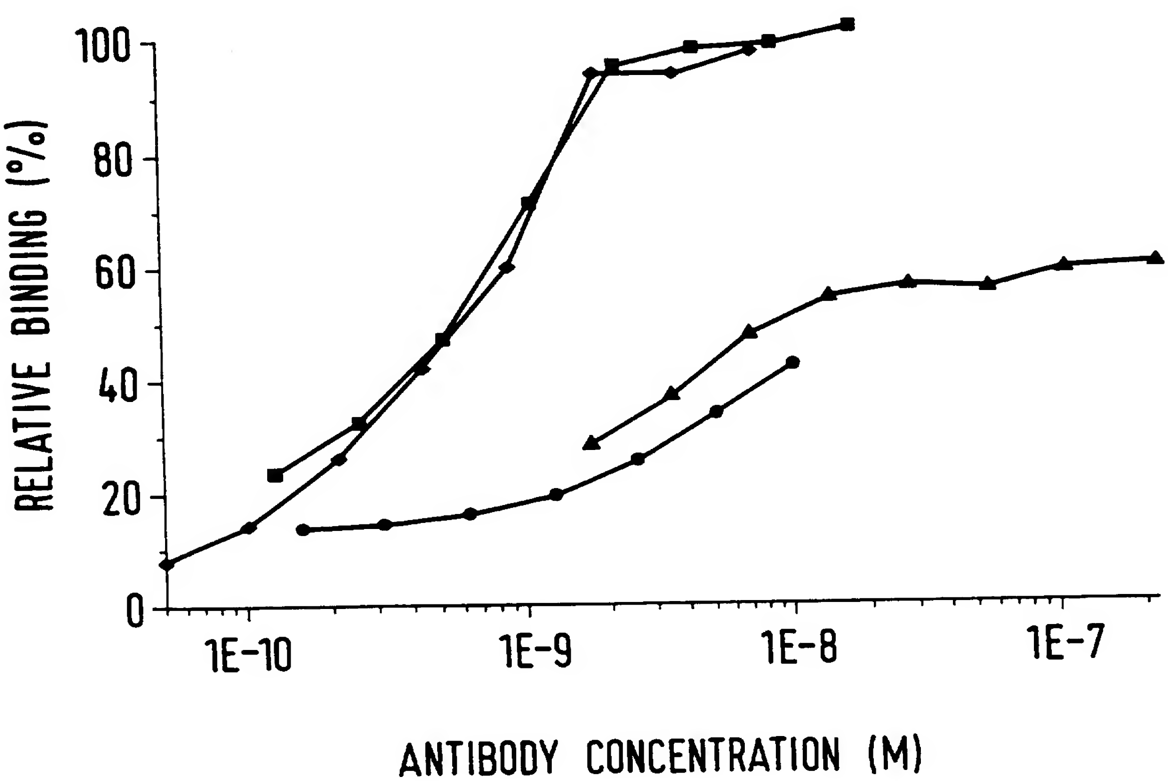

Fig. 7

EFFECT OF VARIOUS HEAVY CHAIN FRAMEWORK SUBSTITUTIONS ON ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY MEDICATED BY CD38 ANTIBODIES

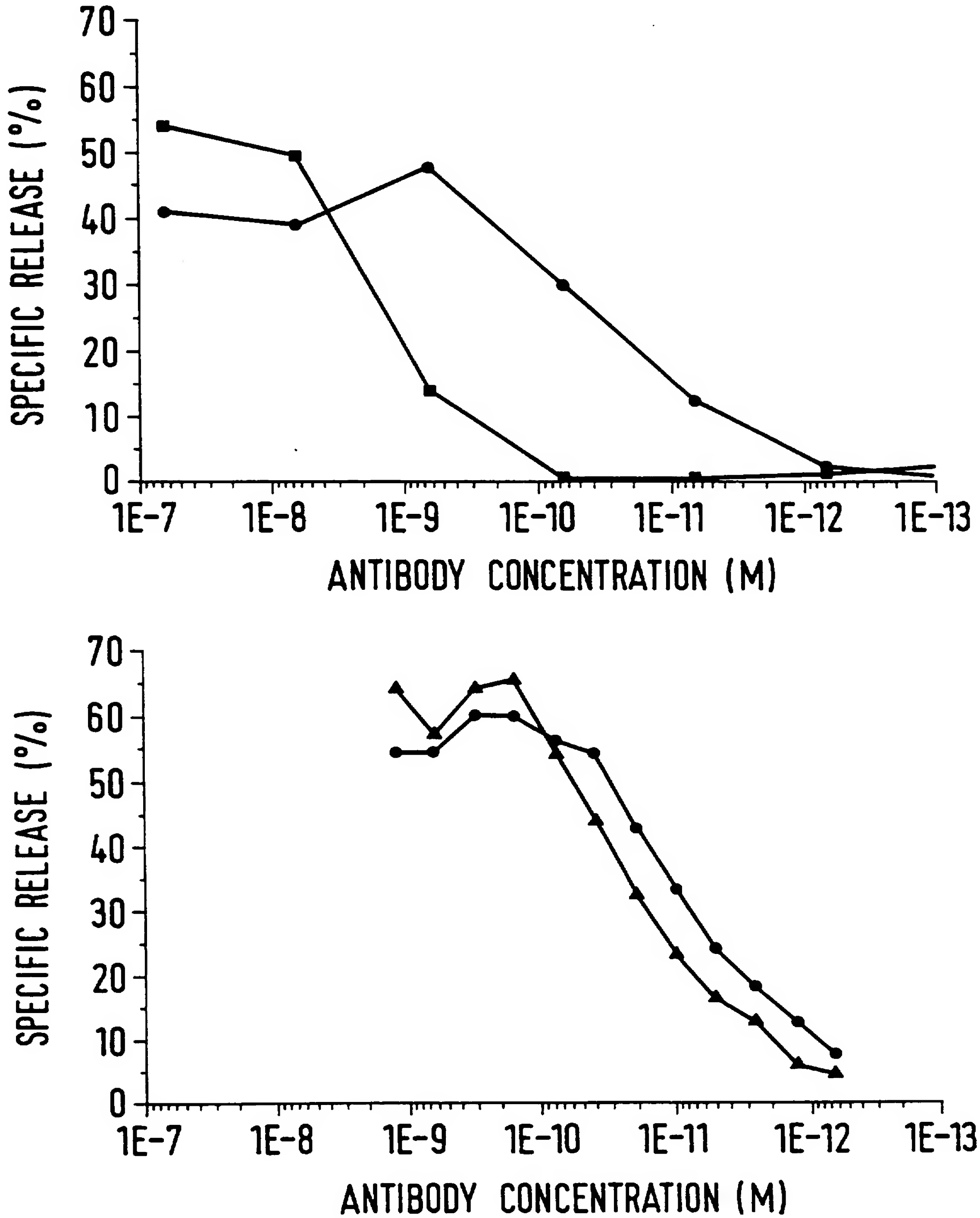

Fig. 8

INTERNATIONAL SEARCH REPORT

Inte[...]onal Application No
PCT/GB 95/02777

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K16/46 C07K16/28 A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF MOLECULAR BIOLOGY, vol. 235, no. 1, 7 January 1994 LONDON, GB, pages 53-60, XP 000564648 A. CORTI ET AL. 'Idiotope determining regions of a mouse monoclonal antibody and its humanized versions.'	1-5,7
Y	see the whole document ---	9
Y	WO,A,94 17184 (SCHERING CORPORATION ET AL.) 4 August 1994 see examples see claims ---	9

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
27 March 1996	23.04.96
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Nooij, F

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,94 13805 (CELLTECH LTD.) 23 June 1994 see page 12, line 19 - page 13, line 5 see example 1 see claims	1,3,4,7, 8,13,14, 16,18
X	WO,A,91 09967 (CELLTECH LTD.) 11 July 1991 see table 2 see examples see claims	1,3,5,7, 13-18
X	WO,A,94 09136 (KETTOCK LODGE, CAMPUS 2) 28 April 1994 see claims	1,3,5,7, 13
X	JOURNAL OF MOLECULAR BIOLOGY, vol. 224, no. 2, 20 March 1992 LONDON, GB, pages 487-499, XP 000564649 J. FOOTE ET AL. 'Antibody framework residues affecting the conformation of the hypervariable loops.' see abstract see table 2	1,3,5-7, 11,13
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 89, no. 10, 15 May 1992 WASHINGTON, DC, USA, pages 4285-4289, XP 000275844 P. CARTER ET AL. 'Humanization of an anti-p185HER2 antibody for human cancer therapy.' see abstract see table 1	1,3,5,7, 13-18
A	EP,A,0 481 790 (THE WELLCOME FOUNDATION) 22 April 1992 see claims	1-18
P,X	THE JOURNAL OF IMMUNOLOGY, vol. 155, no. 2, 15 July 1995 BALTIMORE, MD, USA, pages 925-937, J. ELLIS ET AL. 'Engineered anti-CD38 monoclonal antibodies for immunotherapy of multiple myeloma.' see the whole document	1-18

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1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,O, X	IMMUNOLOGY, vol. 83, suppl. 1, 5 - 7 December 1994 OXFORD, GB, page 70 XP 000565931 J. ELLIS ET AL. 'Characterisation of a humanised monoclonal anti-CD38 antibody.' see abstract -----	1-18

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB95/02777

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 14,15
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 14 and 15 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 95/02777

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9417184	04-08-94	AU-B-	6123894	15-08-94
WO-A-9413805	23-06-94	AU-B-	5656894	04-07-94
		EP-A-	0628077	14-12-94
		GB-A-	2278357	30-11-94
		JP-T-	7504334	18-05-95
WO-A-9109967	11-07-91	AT-T-	129017	15-10-95
		AT-T-	124459	15-07-95
		AU-B-	664801	30-11-95
		AU-B-	6461294	22-12-94
		AU-B-	646009	03-02-94
		AU-B-	6974091	24-07-91
		AU-B-	649645	02-06-94
		AU-B-	7033091	24-07-91
		AU-B-	631481	26-11-92
		AU-B-	7048691	24-07-91
		BG-B-	60462	28-04-95
		CA-A-	2037607	07-09-92
		CA-A-	2046904	22-06-91
		DE-D-	69020544	03-08-95
		DE-T-	69020544	18-01-96
		DE-D-	69022982	16-11-95
		EP-A-	0460167	11-12-91
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		EP-A-	0620276	19-10-94
		EP-A-	0626390	30-11-94
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		WO-A-	9109968	11-07-91
		GB-A,B	2246781	12-02-92
		GB-A,B	2246570	05-02-92
		GB-A,B	2268744	19-01-94
		GB-A,B	2268745	19-01-94
		JP-T-	4505398	24-09-92
		JP-T-	4506458	12-11-92
		JP-T-	5500312	28-01-93
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 95/02777

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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		AU-B-	8591491	30-04-92
		CA-A-	2053585	18-04-92
		JP-A-	6090752	05-04-94
		NZ-A-	240249	25-03-94
		ZA-A-	9108248	16-04-93